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A BIOAUGMENTATION APPROACH TO PESTICIDE BIOREMEDIATION: PESTICIDE DEGRADATION AND CROP PROTECTION

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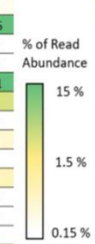
BACKGROUND

- A pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating pests [1].
- The main function of pesticides is crop protection. It is estimated that diseases, insects and weeds destroy between 31-42% of all crops produced worldwide, and the annual worldwide crop loss from plant diseases is about 220 billion dollars [2].
- Many organisms can degrade pesticides. Eukaryotic organisms, like many plants, animals, or fungi, use this ability to neutralize their harmful effects, while bacteria typically break down the pesticide to assimilate its nutrients. Bacteria are accounted for most of the pesticide biodegradation. Their ability to transfer genes horizontally, combined with a fast evolution rate, enables them to quickly adapt and extend the biodegradation potential of a new mutant to a whole bacterial community [3].

15 Most Abundant Genus	Sludge	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Pseudomonas	0.00	63.98	55.97	53.96	54.97	42.57	0.33
Hyphomicrobiaceae	0.00	0.01	0.20	1.14	11.93	17.51	31.06
Methyloceanibacter	50.84	0.15	0.10	0.14	0.01	0.06	0.00
Streptococcus	0.00	9.83	13.67	9.61	0.78	0.52	0.00
Xanthobacteraceae	0.00	0.00	0.01	0.06	0.23	1.33	31.61
Rhizobiaceae	0.00	0.53	1.30	3.06	10.65	4.66	4.72
Shinella	0.00	1.90	4.93	6.61	7.37	1.77	0.10
Parapedobacter	0.00	2.17	3.02	3.59	1.39	9.73	0.66
Vagococcus	0.00	8.06	5.30	2.92	0.14	0.85	0.01
Sphingomonadaceae	0.00	1.33	2.15	4.57	4.00	1.49	0.93
Lactococcus	0.00	3.37	2.81	1.75	0.24	1.25	0.00
Gammaproteobacteria	8.30	0.00	0.01	0.02	0.00	0.00	0.00
Brevundimonas	0.00	2.42	2.65	2.24	0.47	0.22	0.03
Devosiaceae	0.00	1.13	2.02	2.19	0.91	0.31	0.96
Rhodopseudomonas	0.00	0.00	0.00	0.00	0.01	0.27	6.31



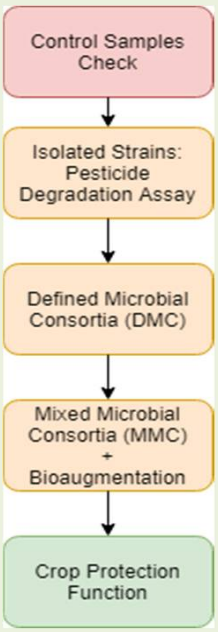
List of the 15 most abundant genus in the enrichment, and their abundance (%). The enrichment strategy lasted for 11 weeks, following 3 initial 1 week steps, a 4th 2 weeks step and two weeks 5th and 6th step.
It can be observed that a major change occurred between step 5 and step 6 of the enrichment, with the variability decreasing, and some species than were predominant until the fifth step (ex. Pseudomonas) almost disappear in the 6th step.
Aside from the decrease in Pseudomonas, there is a high increase in the Hyphomicrobiaceae and Xanthobacteraceae genera.
Since the 5th step and the 6th step of enrichment are very different in variability, both will be utilized in the experiments.

RESEARCH QUESTIONS

1. Is it possible to find efficient pesticide degrading organisms in contaminated soils and sludge ?
2. Do pesticide-degrading organisms also exhibit crop protection function ?
3. Can pesticide-degrading microorganisms improve plant growth and prevent toxicity of pesticides present ?

METHODS

- **Enrichment** of environmental samples in M9 media supplemented with pesticides (Dicamba, Dimethoate, Malathion).
- **Isolation** of strains at the end of the enrichment.
- **Pesticide Degradation Assay**: Degradation efficiency of the previously isolated strains is assessed through a 3 week experiment. The organisms are incubated at 30 degrees C in M9 media supplemented with Dicamba/Dimethoate/Malathion. Determination of the degraded amount is performed through HPLC analysis.
- Development of a **Defined Microbial Consortium** (DMC). The DMC will be formed from the best degraders found from testing the isolated strains.
- **The Crop Protection Function:** the effect of pesticide-degrading bacteria on plant growth parameters and their ability to control fungal plant pathogens will be tested in plant assays.

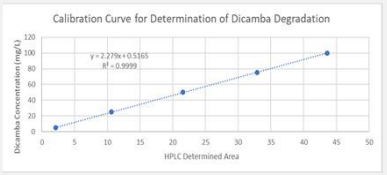


PRELIMINARY RESULTS

Bacterial Degradation of Dicamba, Dimethoate and Malathion

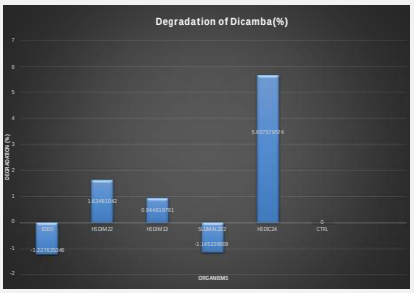
Testing of 6 bacterial isolates in M9 media supplemented with Dicamba 50 mg/L, working volume 20 mL.

Dicamba Preliminary Results



Calibration curve of the HPLC method for the pesticide Dicamba.

The first step of determining the accuracy of the method was performed, obtaining an R square value of 0.9999. Since Dicamba is a herbicide, and it is highly soluble in water, it was chosen as the first pesticide to test.



Degradation of 60 mg/L Dicamba in M9 media. The results have been normalized and calculated through comparison with the control samples. The nomenclature of the organisms is: HS-Hungarian Soil, DIC-isolates from the enrichment performed with Dicamba, DIM-isolates from the enrichment performed with Dimethoate; IDEO- Ideonella sp.; SLU- Sludge samples; MAL- isolates from the enrichment performed with Malathion; In this case, most organisms used were isolated from the Dicamba enrichment.

Dicamba Preliminary Conclusions

Compared to the control sample, one organism degraded 3.87 mg/L of Dicamba,HSDIC24, an isolate that was enriched on Dicamba, while most others seem to have not degraded the herbicide. This represents only the first batch of tested organisms, approximately 60 more isolates shall be tested in order to determine if they can degrade the herbicide Dicamba.

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